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Effects of *Melia azedarach* on nutritional physiology and enzyme activities of the rice leaffolder *Cnaphalocrocis medinalis* (Guenée) (Lepidoptera: Pyralidae)

S. Senthil Nathan *

Department of Environmental Engineering, Chonbuk National University, Jeonju City, Chonbuk 561 756, Republic of Korea
Post Graduate and Research Department of Biotechnology, Vivekanandha College (W), Trichengode, Namakkal, Tamil Nadu 637 205, India

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Abstract

Laboratory assays were done to evaluate the effect of *Melia azedarach* L. (Rutales: Meliaceae) seed extract on nutritional indices and gut enzymes acid phosphatases, alkaline phosphatases, adenosinetriphosphatases, and lactate dehydrogenase of the rice leaffolder (RLF) *Cnaphalocrocis medinalis* (Guenée) (Lepidoptera: Pyralidae). Larvae were fed a treated rice-leaf diet containing the seed extract and their midgut was used for enzyme determination. Laboratory experiments showed that the seed extract suppressed the larval activity of *C. medinalis* even at a low dose. Gross dietary utilization (efficiency of conversion of ingested and digested food) of RLF decreased after ingesting the treated rice-leaf diet. Food consumption, digestion, relative consumption rate, efficiency of conversion of ingested food, efficiency of conversion of digested food, and relative growth rate values declined significantly. As compared to the control, consumption of the extract containing rice-leaf diet resulted in a 69% reduction of the acid phosphatases activity, a 71% reduction of the alkaline phosphatases activity, a 46% reduction of the adenosine triphosphatases activity, and a 52% inhibition of the lactate dehydrogenase activity.

Keywords: Rice leaffolder; Botanical insecticide; *Melia azedarach*; Feeding physiology; Enzyme ACP; ALP; ATPase; LDH

1. Introduction

Developed and developing countries progressing rapidly in agriculture, technology, and industry are introducing various kinds of harmful substance into the biosphere and thus face serious environmental pollution challenges. Chemical pollution by pesticides has been increasing in a large scale due to their vast usage for eradication of various pests and insects and to protect agricultural crops [1]. Consequently, an intensive effort has been made to find alternative methods

* Fax: +82 63 270 2449.
E-mail addresses: senthilkalaidr@hotmail.com, senthil@moak.chonbuk.ac.kr.

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of pest control. Botanical insecticides and microbial pesticides are highly effective, safe, and ecologically acceptable [2].

Botanical insecticides are naturally occurring chemicals extracted from plants. As a consequence of concern about the environmental persistence of synthetic pesticides and their potential toxicity to humans, beneficial insects, and domestic animals, there is renewed interest in natural products to control pests. Naturally occurring biopesticides seem a logical choice for further investigation. Species of Meliaceae and Rutaceae have received much attention due to the fact that they are chemically characterized by triterpenes known as limonoids [3]. Many of these compounds have been demonstrated to affect insect growth and behavior, acting as antifeedants, toxins, and insect growth regulators [4].

The Meliaceae plant family is known to contain a variety of compounds that show insecticidal, antifeedant, growth regulating, and development modifying properties [5–10]. One member of the Meliaceae, known as Chinaberry or Persian lilac tree (*Melia azedarach* L.) (Rutales: Meliaceae) is a deciduous tree that is native to northwestern India and has long recognized for its insecticidal properties but is yet to be wholly analyzed. The effects of compounds, products, and extracts obtainable from *M. azedarach* on insects have been reviewed by Ascher et al. [11]. The antifeedant effects of *M. azedarach* extracts are known for many insects [5,7,8,10–16].

Alkaline phosphatase (ALP, E.C.3.1.3.1) and acid phosphatase (ACP[1] E.C.3.1.3.2) are hydrolytic enzymes, which hydrolyze phosphomonoesters under alkaline or acid conditions, respectively. ALP is primarily found in the intestinal epithelium of animals and its major function is to provide phosphate ions from mononucleotide and ribonucleo-proteins for a variety of metabolic processes. ALP is involved in the transphosphorylation reaction [17]. ATPases are essential for the transport of glucose, amino acids, and other organic molecules. Any impairment in their activity will affect the physiology of the insect gut. These enzymes are located in the midgut, malpighian tubules, muscles, and nerve fibers of the lepidopteran insects [18]. The midgut has the highest ALP and ACP activity as compared to other tissues. The ALP and ACP activities are low during the larval moulting stage and increased gradually after moulting [19]. The highest activity appeared before the full-appetite, gluttonous stage of the fourth instar and the lowest activity was found in the mature larval stage [20]. Lactate dehydrogenase (LDH) (EC 1.1.1.28) is an important glycolytic enzyme being present in virtually all tissues [21]; it is also involved in carbohydrate metabolism and has been used as indicative criterion of exposure to chemical stress [20,22].

The rice leaffolder (RLF) *Cnaphalocrocis medinalis* (Guenée) (Lepidoptera: Pyralidae) is a major insect pest of rice (*Oryza sativa* L.) [23]. Outbreaks of serious RLF infestations have been reported in many Asian countries including India, Korea, Japan, China, Malaysia, Sri Lanka, and Vietnam [24–27] The larvae feed by scraping the green mesophyll tissues of rice leaves, thus producing linear pale white stripe damage. The general vigor and photosynthetic ability of an infested rice plant is greatly reduced [23]. Misuse of chemical insecticides has increased RLF populations because the sprayed insecticides reduce populations of natural enemies of RLF and its biological control in the field [28]. Management of this insect pest using synthetic chemicals has failed because of insecticide resistance, pest resurgence, and environmental pollution [29]. Though biological control has an important role to play in modern pest control programmes, it rarely provides a complete solution to any pest problem. The practical problem of inadequate documentation makes it a challenge to present biological control alternatives. Consequently, the trend has shifted to biocides [24]. The objective of this research is to define the effects of *M. azedarach* extracts on nutritional indices and activities of gut enzymes in the rice leaffolder.

[1] *Abbreviations used:* CI, consumption index; RGR, relative growth rate; AD, approximate digestibility; ECI, efficiency of conversion of ingested food; ECD, efficiency of conversion of digested food; ACP, acid phosphatases; ALP, alkaline phosphatases; ATPase, adenosine triphosphatases; LDH, lactate dehydrogenase; EC, effective concentration.

2. Materials and method

2.1. Laboratory mass culture of Cnaphalocrocis medinalis

Cnaphalocrocis medinalis larvae were collected from paddy fields in and around Erode district, Tamil Nadu, India. Larvae were reared in a greenhouse on potted rice plants covered with mesh sleeves at 28 ± 2 °C, 65% relative humidity, with a 14:10 light:dark cycle. Rice plants were grown in earthenware pots, 18 cm tall with a 20 cm diameter top. Each pot held 15 plants and gave about 62 tillers [23]. The pots were placed in about 10 cm of water in a metal tray in the greenhouse. The culture was initiated with partly grown larvae from the field. Thereafter, newly hatched larvae were placed on plants of the rice variety IR20, about 50-days old.

After pupation, adults emerged on plants in the sleeves. To maintain the culture, 12 female and 13 male moths were placed in an oviposition cage containing one potted plant. The moths were fed with 10% sucrose solution fortified with a few drops of vitamin mixture (Multidec drops, Ashok Pharmaceuticals, Chennai-24, India) to enhance oviposition. After two days, the potted plants were removed from the oviposition cage. The leaf portions containing the eggs were clipped and placed on moist filter paper in a Petri dish. These eggs were used to maintain the culture [24].

2.2. Methanolic extracts of leaves of M. azedarach

Methanolic extracts were prepared from ripe fruits of *M. azedarach* collected from trees in undisturbed natural forests of the Kolli hills, Namakkal district, Southern India. *M. azedarach* fruits were manually de-pulped and the kernels (endocarps) were thoroughly washed with distilled water. Extracts of seeds were obtained as follows. The seeds were crushed to fine particle size and shade dried at room temperature. Extraction was carried out according to the procedure of Warthen et al. [30]. In a 1000 ml flask, 100 g of crushed and dried seed materials in 1000 ml of methanol was stirred for 3 h. After leaving the methanolic solution overnight, it was filtered through Whatman No. 40 filter paper. The solid filtration residue was extracted again following an identical procedure, and the two filtrates were combined. The solvent was removed by vacuum evaporation in a rotary evaporator (28 °C ± 2 °C), and a dark red residue from seed was obtained (100 mg/ml). This crude extract was used to prepare stock solution.

2.3. Preparation of stock solution

A known amount (100 mg/ml) of filtered crude extract obtained from the above process was serially diluted to obtain the desired concentration of 0.25, 0.50, 1.0, and 2.0% of *M. azedarach* extract (MAE). One drop of emulsifier (Tween 20, Sigma Chemical) was added to seed extracts to ensure complete miscibility of the material in methanol.

2.4. Bioassays and treatment

Bioassays were performed with first to fifth instars of *C. medinalis* using concentrations of 0.25, 0.5, 1, and 2% of MAE (3 ml in 9 cm^2 rice leaf). Nine control leaves were treated with methanol and air-dried. A minimum of 20 larvae/concentration were used for all the experiments and the experiments were replicated 5 times (total $n = 100$). Larval weight/mortality was recorded after 5 days at 28 °C and 16:8 (L:D) photoperiod and the effective concentration (EC_{50}) was calculated. From the effective concentration, physiological doses were selected for all the experiments. Also a starved group of larvae (control) simultaneously assayed with the treated group.

2.5. Feeding deterrence index

Antifeedant activity was assayed using a leaf-section choice test [25,26,31,32]. In a 15 cm^2 diameter Petri dish lined with a moist filter paper disc, 9 cm^2 long leaf sections from IR20 rice plants were sprayed (5 ml) on both sides with various concentrations of MAE (0.25, 5, 1, and 2%) the filter paper removed after spraying and replaced with new moistened filter paper. Control leaf sections were treated with methanol alone. The leaf sections were dried at room temperature and then fourth instars of *C. medinalis* starved for 4 h were

introduced into each arena lined with moist filter paper containing one treated and one untreated leaf section in alternate positions. Experiments consisted of using two larvae per dish in five replicates (total $n = 10$). Consumption was recorded using a digitizing leaf area meter (Model LI-3000, LI-COR) after 12 h. The index of feeding deterrence (IFD) was calculated as $(C - T)/(C + T) \times 100$ [25,26,31,32] where C is the consumption of control leaf section and T is the treated leaf section.

2.6. Quantitative food utilization efficiency measures

A gravimetric technique was used to determine weight gain, food consumption, and feces produced. All weights were measured using a monopan balance accurate to 0.1 mg. The fresh rice leaves (*Oryza sativa* L.) were sprayed with 0.25, 0.5, 1, and 2% concentrations of MAE and air-dried. The formulations were applied to leaves with a regulator-controlled spray applicator (3 ml). Control leaves were treated with methanol and air-dried. The newly moulted fourth instar larvae were starved for 4 h. After measuring the initial weight of the larvae, they were individually introduced into separate containers. The larvae (10 larvae/concentration, five replicates) (total $n = 50$) were allowed to feed on five leaves of weighed quantities of MAE treated and untreated IR20 rice leaves, for a period of 24 h. The uneaten leaves were removed every 24 h, and replaced with fresh treated leaves, larvae were again weighed. The difference in weight of the larvae gives the fresh weight gained during the period of study. Sample larvae were weighed, oven dried (48 h at 60 °C) reweighed to establish a percentage dry weight of the experimental larvae. The leaves remaining at the end of each day were oven dried and re-weighed to establish a percentage dry weight conversion value to allow for the estimation of diet dry weight. The quantity of food ingested was estimated by subtracting the diet (dry weight) remaining at the end of each experiment from the total dry weight of the diet provided. Feces were collected daily and weighed, then oven dried and re-weighed to estimate the dry weight of excreta. The experiment was continued for four days and observations were recorded every 24 h.

Consumption, growth rates, and post-ingestive food utilization efficiencies (all based on dry weight) were calculated in the traditional manner [25,26,33,34], such as: Consumption index (CI) $= E/TA$, relative growth rate (RGR) $= P/TA$, approximate digestibility, (AD) $= 100(E - F)/E$, efficiency of conversion of ingested food (ECI) $= 100\ P/E$, efficiency of conversion of digested food (ECD) $= 100\ P/(E - F)$, where, A is the mean dry weight of animal during T, E is the dry weight of food eaten, F is the dry weight of feces produced, P is the dry weight gain of insect, and T is the duration of experimental period.

2.7. Preparation of enzyme extract

Two-day-old fourth instars of treated *C. medinalis* were used to quantify the enzyme activities. The method used to prepare the enzyme extract was that of Applebaum [35] and Applebaum et al. [36]. Individuals were anesthetized with cotton pads soaked in ether and the entire digestive tract dissected out in ice-cold insect Ringer's solution. The Malpighian tubules, adhering tissues ,and gut contents were removed. The gut was split into regions, weighed (accuracy in mg) and homogenized for 3 min at 4 °C in ice-cold citrate–phosphate buffer (pH 6.8) using a tissue grinder. Homogenized gut was suspended in ice-cold buffer and made up to 1 ml. The homogenate was centrifuged at 500 rpm for 15 min and the supernatant was used as the enzyme source.

2.8. Estimation of acid (E.C.3.1.3.2) and alkaline phosphatases (E.C.3.1.3.1)

The enzyme assays were carried out as described by Bessey et al. [37]. The buffered substrate was incubated with tissue extract for 30 min. Alkali was added to stop the reaction and to adjust the pH for the determination of the concentration of the product formed. The spectral absorbance of *p*-nitrophenolate was maximal at 310 nm. The molar absorbance of *p*-nitrophenolate at 400 nm is about double that of *p*-nitrophenyl phosphate at 310 nm. On converting the *p*-nitrophenolate into *p*-nitrophenol by acidification, the absorption

maximum is shifted to about 320 nm with no detectable absorption at 400 nm.

2.9. Estimation of adenosine triphosphatases

The specific activity of sodium- and potassium-dependent adenosine triphosphatases (ATPase) in the gut was assayed according to the method described by Shiosaka et al. [38].

The quantity of inorganic phosphorous liberated was assayed according to the method of Fiske and Subbarow [39]. In this method, the protein is precipitated with trichloroacetic acid. The protein-free filtrate is treated with acid molybdate solution and the phosphoric acid formed is reduced by the addition of 1-amino-2-napthol-4-sulfonic acid (ANSA) reagent to produce blue color. The intensity of the color is proportional to the amount of phosphorous present.

2.10. Estimation of lactate dehydrogenase (EC 1.1.1.27)

To standardize volumes, 0.2 ml NAD+ solution was added to the 'test' and 0.2 ml of water was added to the control test tubes, each containing 1 ml of the buffered substrate; 0.01 ml of the sample was also added to the 'test.' Test tube samples were incubated for exactly 15 min at 37 °C and then arrested by adding 1 ml of color reagent (2,4-dinitrophenyl hydrazine reagent) to each tube and the incubation was continued for an additional 15 min. After the contents were cooled to room temperature, 10 ml of 0.4 N NaOH was added to each tube to make the solutions strongly alkaline to maximize development of hydrazine. At exactly 60 s after the addition of alkali to each tube, the intensity of color was measured at 440 nm. Replicated blanks with standards were run through the same procedure. Inclusion of the calculated amount of reduced co-enzyme in the standard makes allowance for the chromogenicity of $NADH_2$ formed in the test. The enzyme activity is expressed as multi-International Units (mIU) per milligram protein per minute [40].

A mIU is defined as the amount of enzyme that is required to catalyze the conversion of 1 μm lactate to pyruvate or pyruvate to lactate per minute per milliliter of the sample under the prescribed assay conditions.

2.11. Statistical analysis

The effective concentration was calculated using probit analysis [41] and values were expressed as the mean of five replicates with standard error. Data from nutritional indices, enzyme activities, weight, and feeding deterrence were subjected to analysis of variance (ANOVA of arcsine square root transformed percentages). Differences between the treatments were determined by Tukey's multiple range test ($P \leqslant 0.05$) [42,43].

3. Results

3.1. Effect of M. azedarach on feeding, larval weight, and larval mortality

Tables 1 and 2 show the feeding deterrence index and weight loss of fourth instar larvae of

Table 1
Antifeedant activities of *M. azedarach* extract against fourth instar larvae of *C. medinalis*

Concentrations (%)	Feeding deterrence index (%) (fourth instar)
Control	0.08 ± 0.006^{e}
0.25	12.3 ± 2.5^{d}
0.50	22.5 ± 3.2^{c}
1.00	56.8 ± 3.8^{b}
2.00	91.6 ± 6.5^{a}

Means standard error (SE) followed by the same letter within columns indicate no significant difference ($P \geqslant 0.05$) in a Tukey test.

Table 2
Fourth instar *C. medinalis* larval weight after treatment with *M. azedarach* extract

Treatments (%)	Mean (±SE) larval weight (mg)
Control	36.4 ± 4.5^{a}
0.25	34.5 ± 4.0^{a}
0.50	28.6 ± 3.0^{ab}
1.00	20.8 ± 2.5^{c}
2.00	16.5 ± 2.0^{cd}

Means standard error (SE) followed by the same letter within columns indicate no significant difference ($P \geqslant 0.05$) in a Tukey test.

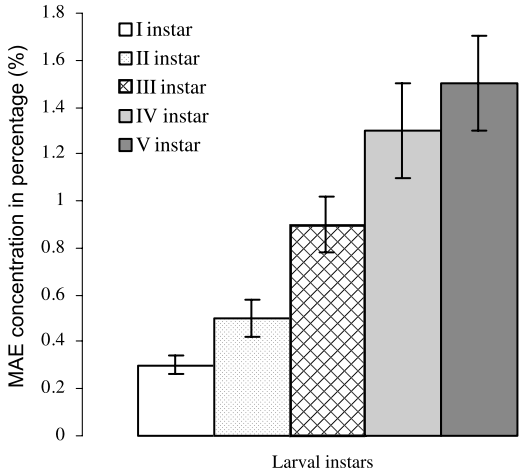

Fig. 1. Effective concentrations (EC_{50}) of MAE against first to fifth instar larvae of *C. medinalis*. Values are (±SE) means of five replicates.

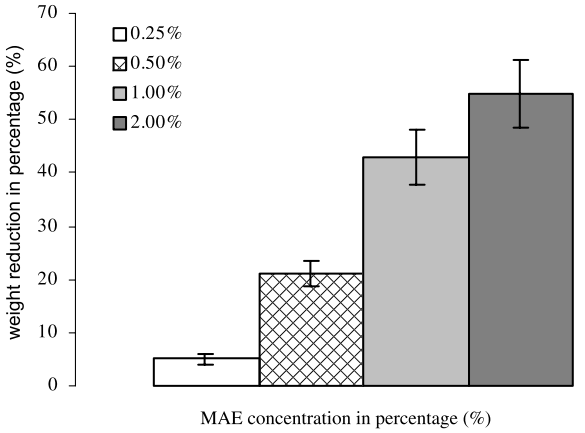

Fig. 2. Percentage reduction of weight in fourth instars larvae of *C. medinalis* after treatment with MAE.

C. medinalis treated with MAE. The lowest averages were achieved at the lowest MAE concentrations (0.25%). As extract concentration increased, the deterrence index also increased in a dose-dependent manner. A deterrence index of 92% was calculated in the 2% treatment of fourth instar (Table 1). An EC_{50} value of against RLF was shown in Fig. 1. First and second instar larvae were more susceptible with least EC_{50} values (Fig. 1). Larval weight decreased in fourth instar larvae due to treatment with MAE. Larval body weight was 36.4 mg in fourth instar in the control. In the 0.25% concentration of MAE, it decreased to 34.5 mg (5%) and was further reduced to 16.5 mg (55%) in the 2% MAE treatment (Table 2, Fig. 2). Higher doses also caused mortality and severe deformities in the larvae, pupae, and adults in a dose-dependent manner (Fig. 3). The larvae were slow in completing the moult and at higher concentrations the larvae died in a failed moult attempt (Fig. 3A).

3.2. Nutritional physiology of *C. medinalis* after treatment with *M. azedarach*

Dietary utilization by *C. medinalis* was severely affected when fed on rice leaves treated with MAE (Table 3). The adverse effects of MAE on the feeding and growth of *C. medinalis* were evident from the nutritional experiment. Furthermore, the consumption and relative growth rates of *C. medinalis* were reduced by MAE. They revealed that the extract acts as a chronic toxin when ingested by larvae. The crude extract, when applied in rice leaf diet, reduced RGR, ECI, ECD, and CI. The absolute growth and RGR of the treated fourth instar larvae remained significantly lower than in the controls (Tables 2 and 3). The RGR in the treated group significantly decreased in insects receiving the higher dose. RGR remained stable when compared with the control in lower-dose fed insects (0.25%). The higher dose treatment of seed extracts affected the nutritional indices to a greater extent. The AD was slightly increased but significant only in higher doses (Table 3). A decrease in the CI, RGR, ECI, and ECD was noticed after treatment with MAE. Day to day consumption and digestion revealed a continuous decrease in food consumption in seed extract fed groups; the lowest ingestion and digestion rates were recorded in the 2% seed extract treatment. Both ECI and ECD recorded the same pattern of change over the course of development in affected group. The value of ECI and ECD was higher in the 2% MAE treatment (Table 3).

3.3. Mid gut enzyme activity of *C. medinalis* after treatment with *M. azedarach*

Differences in acid phosphatase (ACP), alkaline phosphatase (ALP), adenosine triphosphatase

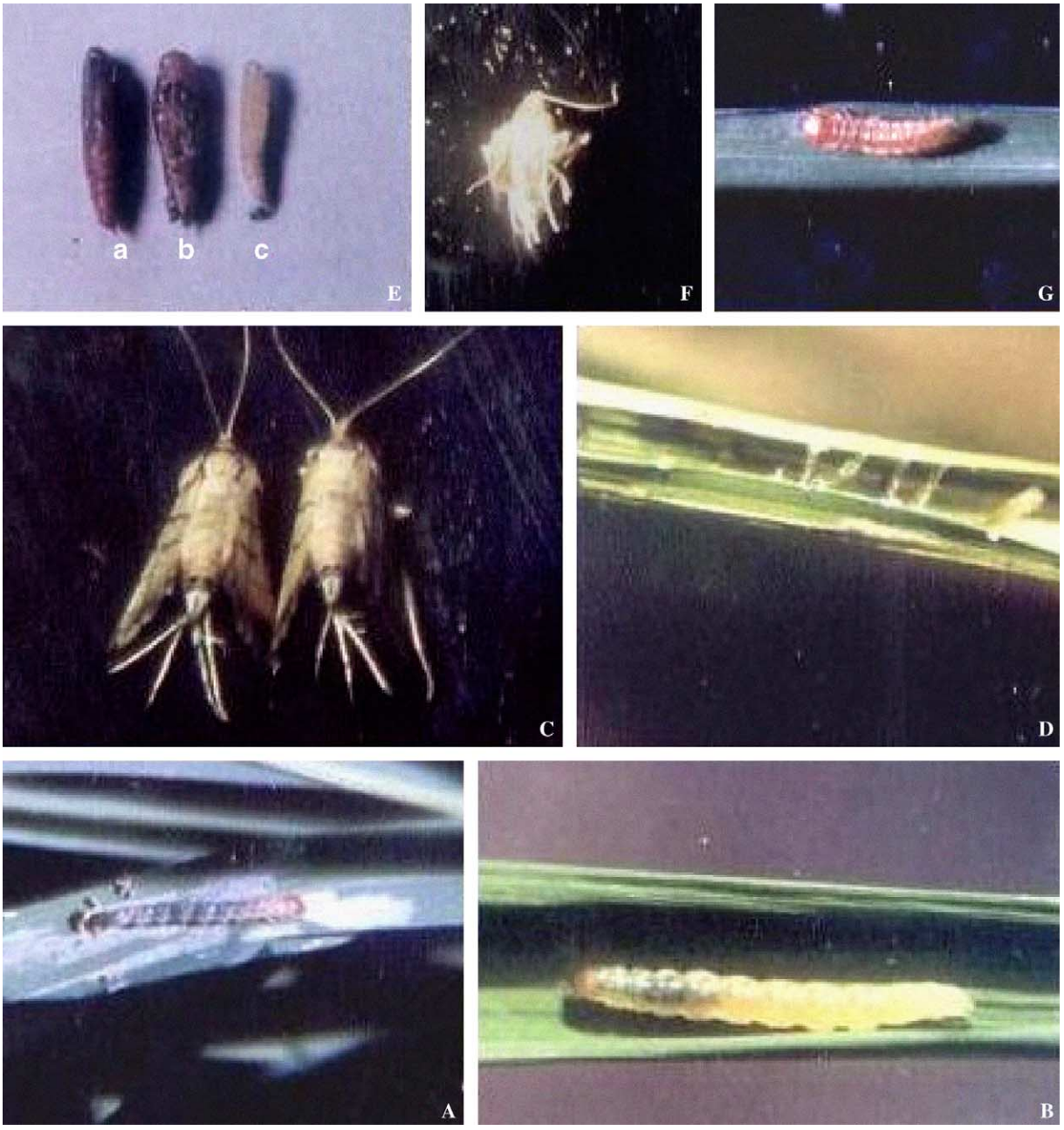

Fig. 3. Physiological and morphological effects MAE on *C. medinalis*. (A) The larvae feed by scraping the green mesophyll tissues of rice leaves; (B) control late fourth instar larva of *C. medinalis*; (C) adult female moths of *C. medinalis*; (D) folding (spinning) behavior of *C. medinalis*; (E) pupal deformities, when larva treated with lower doses of MAE (a, 0.25; b, 0.5; and c, 1%); (F) adult deformities; and (G) moulting disorder (shrinking of larval body) due to 2% MAE treatment.

Table 3
Nutritional indices of fourth instar larvae of *C. medinalis* after treatment with *M. azedarach* extract

Treatments (%)	CI (mg/mg/day)	RGR (mg/mg/day)	AD (%)	ECI (%)	ECD (%)
Control	2.95 ± 0.32^{a}	0.56 ± 0.007^{a}	41.5 ± 4.5^{a}	19.3 ± 2.3^{a}	46.7 ± 5.3^{a}
0.25	2.50 ± 0.30^{ab}	0.45 ± 0.006^{a}	43.5 ± 4.5^{a}	18.0 ± 2.0^{a}	41.5 ± 5.0^{a}
0.50	2.27 ± 0.27^{b}	0.36 ± 0.004^{b}	44.3 ± 4.7^{a}	16.1 ± 1.8^{ab}	36.4 ± 4.0^{b}
1.00	1.83 ± 0.20^{b}	0.25 ± 0.003^{b}	46.7 ± 5.2^{ab}	14.2 ± 1.8^{b}	30.5 ± 3.5^{bc}
2.00	1.14 ± 0.18^{c}	0.13 ± 0.001^{c}	49.5 ± 5.0^{b}	11.7 ± 1.3^{b}	23.7 ± 3.0^{d}

Means standard error (SE) followed by the same letter within columns indicate no significant difference ($P \geqslant 0.05$) in a Tukey test.
Abbreviations used: CI, consumption index; RGR, relative growth rate; AD, approximate digestibility; ECI, efficiency of conversion of ingested food; ECD, efficiency of conversion of digested food.

Table 4
Enzyme activities of fourth instar larvae of *C. medinalis* after treatment with *M. azedarach* extract

Treatments (%)	Acid phosphatase (ACP)*	Alkaline phospahatase (ALP)*	Adenosinse triphospate (ATPase)*	Lactate dehydrogenase (LDH)**
Control	$10.22 \pm 1.73^{a*}$	$16.10 \pm 2.45^{a*}$	$72.32 \pm 6.40^{a*}$	$18.11 \pm 2.42^{a*}$
0.25	8.56 ± 1.26^{a}	14.23 ± 2.00^{a}	68.25 ± 6.15^{a}	15.74 ± 2.18^{a}
0.50	6.23 ± 1.12^{b}	11.86 ± 1.53^{b}	60.14 ± 6.00^{ab}	13.22 ± 2.00^{b}
1.00	5.16 ± 0.95^{b}	6.20 ± 0.96^{c}	47.36 ± 5.63^{c}	11.23 ± 1.43^{b}
2.00	3.12 ± 0.70^{c}	4.54 ± 0.75^{c}	39.20 ± 5.00^{d}	8.64 ± 1.12^{bc}

Means (±SE) followed by the same letters within columns of indicate no significant difference ($P \leqslant 0.05$) in a Tukey test.
* In μmol/mg/h^{-1}.
** mIU/mg/protein/min; ±SE, standard error.

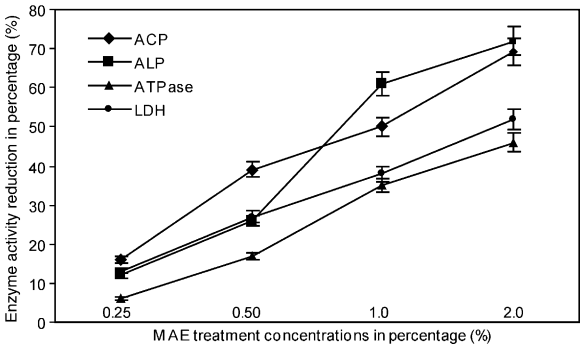

Fig. 4. Percentage reductions of enzyme activities in fourth instar larvae of *C. medinalis* after treatment with MAE.

(ATPase), and lactate dehydrogenase (LDH) activities in the gut between the control and treated fourth instar larvae are shown in Table 4. The maximal suppression of gut enzyme activity was obtained in the 2% MAE treatment in fourth instar larvae. As shown in Fig. 4, ACP, ALP, ATPase, and LDH activities showed maximum reduction after treatment with MAE at 2% (69, 71, 46, and 52%, respectively).

4. Discussion

These tests infer that crude *M. azedarach* seed extracts highly efficacious for the control of the agricultural pest, the RLF *C. medinalis* making them economic alternatives to the purified compounds [10,23,26,27]. The feeding deterrence and nutritional physiology effects of the 2% MAE reported in the present study demonstrate the potential of them for controlling the leaffolder populations. There are species in the Meliaceae plant family that contain antifeedants and insect growth regulators against many insect pests [3,4]. The growth regulatory effect is the most important physiological effect of *M. azedarach* on insects (Fig. 3)[11]. The delay in moulting or development of *C. medinalis* is due to active principles present in *M. azedarach*.

Larvae exposed to all tested concentrations of the MAE differ significantly from the control treatment in terms of feeding. The feeding deterrence index hardly reached 92% in larvae exposed to the extract (2% concentration). This may be explained by the specific chemical constituents in the seed extract,

and especially by the presence of limonoids (triterpenoid), which are common in this species [44].

The present findings showing reduced growth rate during fourth instar, and an extended developmental time in treated larvae, confirms earlier findings [12,14–16]. It may be inferred from the study that the decreased larval growth coupled with lower RGR, which is more likely due to longer retention of food in the gut for maximization of AD to meet the increased demand of nutrients, [25,26]. The results revealed that although the treated larvae were capable of maintaining the AD, they failed to maintain the RGR during larval development (Table 1). AD could not be maintained due to a continuous decline in RGR. The RGR reached it lowest level in the 2% treatment (Table 1). A significant correlation between deterrence and toxicity of ingested secondary plant compounds in locusts has been reported earlier [45]. The consumption of plant extracts resulted in retarded growth and affected the nutritional physiology of the larvae. Furthermore, utilization efficiencies for larvae exposed to *M. azedarach* were reduced significantly. These results are similar to those obtained using neem limonids [28]. The consumption and conversion efficiency were highly correlated with the gut enzyme activity of *C. medinalis*. Plant extracts contain enzyme inhibiting components, which reduce the conversion rate [26,46–48]. In any instars of *C. medinalis* larvae that were fed *M. azedarach* in their diet, growth rates declined as extract concentration increased. This corresponded to a decrease in consumption rate. It is likely that this decrease in consumption rate is due to the antifeedant nature of the extract and this accounts for the majority of the decrease in growth rate [49].

The percentage of reduction in ECI and ECD results from a foodstuff conversion deficiency, which promotes growth, perhaps through a diversion of energy from biomass production into detoxification [49]. Our results show that MAE affected the gut physiology of *C. medinalis* at several doses. Due to the inhibitory effect on the activity of gut enzymes together with the low food consume, the weight of treated larvae was affected. Our data support the hypothesis that changes in metabolism and decreases in the gut enzyme activity in individuals treated with Meliaceous plant compounds indicate that there may be effects on enzyme titers and activities [24,45–48]. Feeding is necessary for the stimulation of digestive enzyme activities [50] and may have interfered with the enzyme–substrate complex thus affecting the peristaltic movement of the gut [51,52], a phenomenon that was very clear observed by the decrease of fecal pellet production in the *M. azedarach* treatment [10].

Similar results were also seen with neem seed kernel extract and pure neem limonoids on RLF [25–29]. Plant allelochemicals may be quite useful in increasing the efficacy of biological control agents because plants produce a large variety of compounds that increase their resistance to insect attack [53–55]. Biopesticides of plant origin are given new importance in recent years for use against several insect species including leaffolder management [2,55]. One of the reasons for their increased usage could be that compounds of plant origin are safer for humans and the environment.

From results of this study, antifeedant substances from *M. azedarach* could play an important role for managing RLF. The results of this study indicate that natural plant products have growth inhibition, antifeedant effect, and probably some toxic effects on harmful insects, hence further investigations should be carried out. The primary effect of these products is to prevent insect feeding and therefore to protect rice plants from severe defoliation. Generally, neem extracts or neem-based insecticides are effective against RLF larvae with significant lethal and antifeedant effects accompanied by significant reduction in food consumption [5,25,26]. However, experimental observations indicated that the larvae died in first and second instars, and the larvae were able to cause considerable foliage damage when older and larger instars were treated [27]. The present data show that MAE was toxic to all larval instars. Therefore, these extracts should be applied as early as possible when the insects are eggs, neonates, or second instar larvae in order to prevent economically significant foliar damage under field conditions. Protection from RLF is critical during last decade depending on the region [29,32]. Therefore, during such a period, plant extracts such as *M. azedarach* seed extracts could be an effective alternative to

conventional synthetic insecticides for the control of RLF, the use of plant extracts or botanical pesticides may play a more prominent role in integrated pest management (IPM) programmes in the future.

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